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1: Transgenic Res. 2005 Jun;14(3):237-49.

Process development and economic evaluation of recombinant human lactoferrin expressed in rice grain.

Nandi S, Yalda D, Lu S, Nikolov Z, Misaki R, Fujiyama K, Huang N.

Ventria Bioscience, 4110 North Freeway, Sacramento, CA 95834, USA.

In this paper, we show that recombinant human lactoferrin (rhLF) has been stably expressed at 0.5% brown rice flour weight for nine generations. Process development indicates that rhLF can be efficiently extracted from rice flour in 20 mM phosphate buffer (pH 7.0) containing up to 0.5 M NaCl and at a ratio of 1 kg flour to 10 L buffer. After solid/liquid separation, the extract can then be loaded directly onto an ion-exchange column and rhLF can be eluted using 0.8 M NaCl. The resulting rhLF is about 95% pure. A range of biochemical and biophysical analyses were carried out and results indicated that the purified rhLF was identical to its native human counterpart other than its glycosylation. Economic analysis shows that at 600 kg/year scale, the cash cost to produce 1 g of rhLF of pharmaceutical grade is US$ 5.90. Analysis also indicates that the expression level has profound impact on costs related to planting, milling, extraction and purification, thus high level expression of recombinant protein in plants is one of the key parameters for the success of plant made pharmaceuticals.

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Transcription of genes for conglutin gamma and a leginsulin-like protein in narrow-leafed lupin.

Ilgoutz SC, Knittel N, Lin JM, Sterle S, Gayler KR.

Department of Biochemistry and Molecular Biology, University of Melbourne, Parkville, Victoria, Australia.

The expression of genes encoding conglutin gamma and a leginsulin-like protein has been examined in narrow-leafed lupin, Lupinus angustifolius L. Conglutin gamma is a homologue of basic 7S globulin (Bg), the insulin and leginsulin binding protein from soybean. Accumulation of conglutin gamma mRNA, as assessed by northern assays and reverse-transcription PCR, was tightly regulated both spatially and temporally in lupin plants and was detected almost exclusively in developing seeds. Similar tissue and temporal specificity was demonstrated when 1.8 kb of the promoter region from the conglutin gamma gene was used to drive the expression of a beta-glucuronidase reporter gene in transgenic plants. In stably transformed tobacco the conglutin gamma promoter produced strong, temporally regulated and seed-specific expression of the reporter gene which was localised to the embryo tissues and to a layer of cells adjacent to the seed coat. A truncated 0.29 kb promoter fragment produced much reduced levels of expression and a loss of embryo specificity. Leginsulin-like mRNA was similarly detected in lupins only in developing seeds. The leginsulin-like gene detected in L. angustifolius showed 96% sequence identity to leginsulin from soybean within the 280 bp region amplified from lupin by PCR. The results demonstrate that both components of a Bg-leginsulin putative signal transduction pathway are present in the seeds of lupin.

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